Nautilus Environmental

Chronic Toxicity Test Results for the Carlsbad Desalination Plant

❖ Sample ID: Daily M-001
Sample Collection Date: February 22, 2017

Prepared for: **IDE AMERICAS, Inc.**
4590 Carlsbad Boulevard
Carlsbad, CA 92008

Prepared by: **Nautilus Environmental**

Submitted: **March 10, 2017**

Data Quality Assurance:

- Nautilus Environmental is accredited in accordance with NELAP by the State of Oregon Environmental Laboratory Accreditation Program (Certificate No. 4053). It is also certified by the State of California Department of Health Services Environmental Laboratory Accreditation Program (Certificate No. 1802) and the State of Washington Department of Ecology (Lab ID C552).
- All data have been reviewed and verified.
- All test results have met minimum test acceptability criteria under their respective EPA protocols, unless otherwise noted in this report.
- All test results have met internal Quality Assurance Program requirements.

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Results verified by: Adrienne Cibor

EXECUTIVE SUMMARY

DAILY CHRONIC TOXICITY TESTING

CARLSBAD DESALINATION PLANT – FEBRUARY 2017

ORDER NO. R9-2006-0065; NPDES NO. CA0109223

Sampling Date: **February 22, 2017**

Test Date: **February 23, 2017**

Sample ID: **Daily M-001 Effluent (Shutdown Period)**

Effluent Limitation: **16.5 TU_c**

Results Summary:

Bioassay Type: Urchin Fertilization	Test Date	Effluent Test Results		Effluent Limitation Met? (Yes/No)
		NOEC	TU_c	
	2/23/2017	15	<6.67	Yes

INTRODUCTION

A discharge sample was collected in February 2017 for the Poseidon Resources (Channelside) LLC, Carlsbad Desalination Project (CDP) permit for chronic toxicity monitoring purposes. The discharge sample was collected from the CDP M-001 discharge monitoring point during a time of plant shut down. Daily chronic toxicity testing for the effluent sample was conducted during this time according to the permit that was adopted in 2006 (Order No. R9-2006-0065). Bioassay testing was conducted at the Nautilus Environmental (Nautilus) laboratory in San Diego, California on February 23, 2017 using the purple urchin (*Strongylocentrotus purpuratus*) chronic fertilization test.

MATERIALS AND METHODS

The composite sample was collected on February 22, 2017. Sample collection and delivery were performed by IDE Americas, Inc. (IDE) personnel. Following arrival at Nautilus, an aliquot of the water sample was poured off and the following water quality parameters were measured: pH, dissolved oxygen (DO), temperature, salinity, alkalinity, and total chlorine. A summary of the sample collection and receipt information is provided in Table 1, and water quality parameters measured upon receipt at Nautilus are presented in Table 2. Testing was conducted in accordance with the protocols described in USEPA 1995, and the methods are summarized in Table 3.

Table 1. Sample Information

Client/Project: IDE Americas, Inc./Carlsbad Desalination Plant

Sample ID: Daily M-001

Monitoring Period: February 2017 (shutdown period)

Sample Material: Facility Effluent

Sampling Method: Composite

Sample Collection Date, Time: 2/22/17, 12:00

Sample Receipt Date, Time: 2/22/17, 13:25

Table 2. Water Quality Measurements for the Daily M-001 Sample upon Receipt

Sample Collection Date	pH	DO (mg/L)	Temp (°C)	Salinity (ppt)	Alkalinity (mg/L as $CaCO_3$)	Total Chlorine (mg/L)
2/22/17	7.80	8.0	3.2	31.5	111	< 0.02

Table 3. Echinoderm Fertilization Chronic Bioassay Specifications

Test Date, Times:	2/23/17, 14:42 through 15:22
Test Organism:	*Strongylocentrotus purpuratus* (purple sea urchin)
Test Organism Source:	Field-collected locally (Point Loma, CA)
Lab Control/Dilution Water:	Natural seawater (source: Scripps Institution of Oceanography (SIO) inlet), 34±2 parts per thousand (ppt); 20-µm filtered
Test Concentrations:	2.5, 5.0, 6.06, 10, and 15 percent unadjusted M-001 sample; lab control.
Number of Replicates, Organisms per Replicate:	5 replicates, 2000 eggs per replicate. Sperm to egg ratio determined before each test with a preliminary rangefinding test.
Test Chamber Type, Volume per Replicate:	Glass scintillation vial containing 10 mL of test solution
Protocol Used:	EPA/600/R-95/136, 1995 West Coast Marine Chronic
Test Type:	Fertilization; 20-min sperm exposure to effluent followed by a 20-min fertilization period
Acceptability Criteria:	Mean fertilization ≥70% in the control, and percent minimum significant difference (PMSD) value <25%
Reference Toxicant Testing:	Copper chloride
Statistical Analysis Software:	CETIS™, version 1.8.7.20

Statistical analyses were conducted using EPA flowchart specifications as outlined in the test guidance manual (USEPA 1995). Organism performance in the sample dilution series was compared to that observed in the laboratory control exposure. Results were used to calculate the No Observed Effect Concentration (NOEC) and chronic toxic unit (TU_c) values.

Results were also analyzed using the USEPA's Test of Significant Toxicity (TST) approach specified in National Pollutant Discharge Elimination System Test of Significant Toxicity Implementation Document (USEPA 2010). Notably, the California State Water Resources Control Board (SWRCB) published a Draft Policy for Toxicity Assessment and Control (SWRCB 2012), which includes the TST as an alternative method to evaluate toxicity data. This approach applies a modified t-test that takes into account both the statistical power of the test and the magnitude of biological effects in determining the presence of a response. For this sample, the in-stream waste concentration (IWC) is 6.06 percent unadjusted effluent, and results are reported as "Pass" if a sample is considered non-toxic at the IWC according to the TST calculation, or "Fail" if considered toxic at the IWC according to the TST. As the TST is not included in the CDP permit at this time, the TST analysis was performed for comparison purposes only.

RESULTS

There were no statistically significant decreases from control in the fertilization rate of any of the sample concentrations tested, resulting in a no observed effect concentration (NOEC) of 15 percent effluent and a chronic toxic unit (TU_c) less than 6.67. The TU_c result was within the permit limit of 16.5. Additionally, the 6.06 percent concentration was not statistically significant using to the TST calculation, which indicates a non-toxic result in the critical concentration according to the TST. Statistical results are summarized in Table 4, and detailed test results are summarized in Table 5. Raw test data and full statistical analyses can be found in Appendix A. Sample receipt information and copies of the chain-of-custody form are in Appendices B and C, respectively.

Table 4. Statistical Results for Daily M-001 Purple Urchin Fertilization Testing

Sample ID	NOEC (% sample)	LOEC (% sample)	EC_{50} (% sample)	TU_c value (toxic units)	TST Result (Pass/Fail)	Percent Effect
Daily M-001	15	>15	>15	<6.67	Pass	-1.9

NOEC = No Observed Effect Concentration
LOEC = Lowest Observed Effect Concentration
EC_{50} = Concentration expected to cause an adverse effect to 50 percent of the test organisms
TU_c = Chronic Toxic Unit: 100÷NOEC
TST: Pass = sample is non-toxic at the IWC according to the TST calculation; Fail = sample is toxic at the IWC according to the TST calculation. The TST analysis is not in the existing CDP permit; TST analysis is included here for comparison purposes only.
Percent effect (PE) from control is calculated as: PE= ((mean response in control-mean response in the IWC)/mean response in control) *100. A negative PE results when organism performance in the sample is greater than that in the control.

Table 5. Detailed Results of Purple Urchin Fertilization Testing for the Daily M-001 Sample

Test Concentration (% Effluent)	Mean Percent Fertilization
Lab Control	82.8
2.5	83.6
5.0	85.6
6.06	84.4
10	83.4
15	82.2

QUALITY ASSURANCE

The sample was received on the same day as collection within the appropriate temperature range, and the test was initiated within the 36-hour holding time. Additionally, the PMSD value, which is a measure of test variability, was within the acceptable limits. Statistical analyses followed USEPA flowchart selections and the dose-response relationship was reviewed to ensure the reliability of the data. Based on the dose response observed during testing, the calculated effect concentrations reported are deemed reliable. Additionally, appropriate alpha levels were used for statistical analyses according to the TST Implementation Document guidelines (USEPA 2010).

Results for the concurrent reference toxicant test used to monitor laboratory performance and test organism sensitivity met all test acceptability criteria. The median effect (EC_{50}) value calculated for this test was within two standard deviations (2SD) above the historical mean for our laboratory, indicating organisms were of typical sensitivity to copper. Results for the reference toxicant test are summarized in Table 6 and presented in full in Appendix D. A list of qualifier codes can be found in Appendix E.

Table 6. Urchin Fertilization Reference Toxicant Test Results

Test Date	EC_{50} (µg/L Copper)	Historical Mean EC_{50} ±2 SD (µg/L Copper)	CV (%)
2/23/17	23.8	43.0 ± 22.6	26.3

EC_{50} = Concentration expected to cause an adverse effect to 50 percent of the test organisms
Historical Mean EC_{50} ± 2 SD = Mean of historical test results plus or minus two standard deviations
CV = Coefficient of Variation

REFERENCES

California State Water Resources Control Board (SWRCB) 2012. Draft Policy for Toxicity Assessment and Control. June 2012. Sacramento, CA.

Tidepool Scientific Software. 2000-2013. CETIS™ Comprehensive Environmental Toxicity Information System Software, Version 1.8.7.20

USEPA. 1995. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms. EPA/600/R-95/136.

USEPA. 2010. National Pollutant Discharge Elimination System Test of Significant Toxicity Implementation Document. EPA/833/R-10/003. June 2010.

Appendix A

Test Data and Statistical Analyses

CETIS Summary Report

Report Date: 06 Mar-17 18:51 (p 1 of 1)
Test Code: 1702-S183 | 11-3910-8922

Echinoid Sperm Cell Fertilization Test 15C — Nautilus Environmental (CA)

Batch ID:	04-4636-4863	Test Type:	Fertilization	Analyst:	
Start Date:	23 Feb-17 14:42	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Natural Seawater
Ending Date:	23 Feb-17 15:22	Species:	Strongylocentrotus purpuratus	Brine:	Not Applicable
Duration:	40m	Source:	Pt. Loma	Age:	

Sample ID:	05-3767-0046	Code:	17-0301	Client:	IDE
Sample Date:	22 Feb-17 12:00	Material:	@ ~~Seawater~~ Facility Effluent	Project:	Carlsbad Desal Plant
Receive Date:	22 Feb-17 13:25	Source:	IDE Americas, Inc.		
Sample Age:	27h (3.2 °C)	Station:	Daily M-001		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
13-2403-4587	Fertilization Rate	15	>15	NA	6.92%	< 6.667	Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
07-8967-1481	Fertilization Rate	EC25	>15	N/A	N/A	<6.667	Linear Interpolation (ICPIN)
		EC50	>15	N/A	N/A	<6.667	

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
07-8967-1481	Fertilization Rate	Control Resp	0.828	0.7 - NL	Yes	Passes Acceptability Criteria
13-2403-4587	Fertilization Rate	Control Resp	0.828	0.7 - NL	Yes	Passes Acceptability Criteria
13-2403-4587	Fertilization Rate	PMSD	0.06924	NL - 0.25	No	Passes Acceptability Criteria

Fertilization Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	5	0.828	0.7804	0.8756	0.77	0.86	0.01715	0.03834	4.63%	0.0%
2.5		5	0.836	0.7962	0.8758	0.8	0.87	0.01435	0.03209	3.84%	-0.97%
5		5	0.856	0.7894	0.9226	0.77	0.91	0.024	0.05367	6.27%	-3.38%
6.06		5	0.844	0.7954	0.8926	0.81	0.91	0.01749	0.03912	4.63%	-1.93%
10		5	0.834	0.8114	0.8566	0.81	0.85	0.008124	0.01817	2.18%	-0.72%
15		5	0.822	0.7981	0.8459	0.8	0.85	0.008602	0.01924	2.34%	0.72%

Fertilization Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.77	0.86	0.84	0.86	0.81
2.5		0.82	0.82	0.8	0.87	0.87
5		0.91	0.86	0.85	0.77	0.89
6.06		0.84	0.81	0.82	0.91	0.84
10		0.85	0.84	0.85	0.81	0.82
15		0.81	0.82	0.8	0.83	0.85

@ QC 18 Ac 3/6/17

Echinoid Sperm Cell Fertilization Test 15C — **Nautilus Environmental (CA)**

Analysis ID: 13-2403-4587 **Endpoint:** Fertilization Rate **CETIS Version:** CETISv1.8.7
Analyzed: 06 Mar-17 18:51 **Analysis:** Parametric-Control vs Treatments **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	6.92%	15	>15	NA	6.667

Dunnett Multiple Comparison Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		2.5	-0.3348	2.362	0.073	8	0.9134	CDF	Non-Significant Effect
		5	-1.322	2.362	0.073	8	0.9935	CDF	Non-Significant Effect
		6.06	-0.7256	2.362	0.073	8	0.9653	CDF	Non-Significant Effect
		10	-0.2163	2.362	0.073	8	0.8893	CDF	Non-Significant Effect
		15	0.2945	2.362	0.073	8	0.7321	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.007906956	0.001581391	5	0.6542	0.6612	Non-Significant Effect
Error	0.05801304	0.00241721	24			
Total	0.06592		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	6.506	15.09	0.2600	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9795	0.9031	0.8126	Normal Distribution

Fertilization Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.828	0.7804	0.8756	0.84	0.77	0.86	0.01715	4.63%	0.0%
2.5		5	0.836	0.7962	0.8758	0.82	0.8	0.87	0.01435	3.84%	-0.97%
5		5	0.856	0.7894	0.9226	0.86	0.77	0.91	0.024	6.27%	-3.38%
6.06		5	0.844	0.7954	0.8926	0.84	0.81	0.91	0.01749	4.63%	-1.93%
10		5	0.834	0.8114	0.8566	0.84	0.81	0.85	0.008124	2.18%	-0.72%
15		5	0.822	0.7981	0.8459	0.82	0.8	0.85	0.008602	2.34%	0.72%

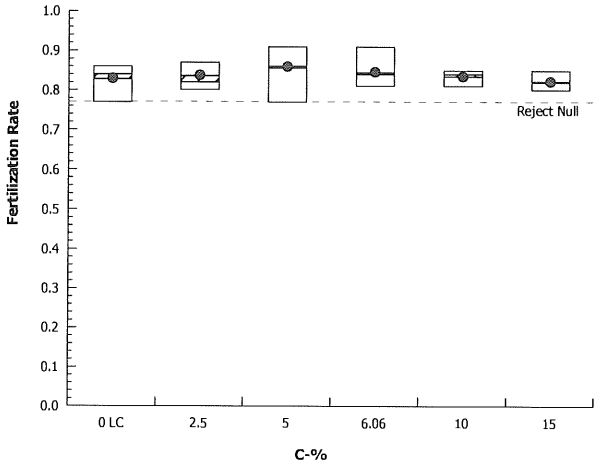
Angular (Corrected) Transformed Summary

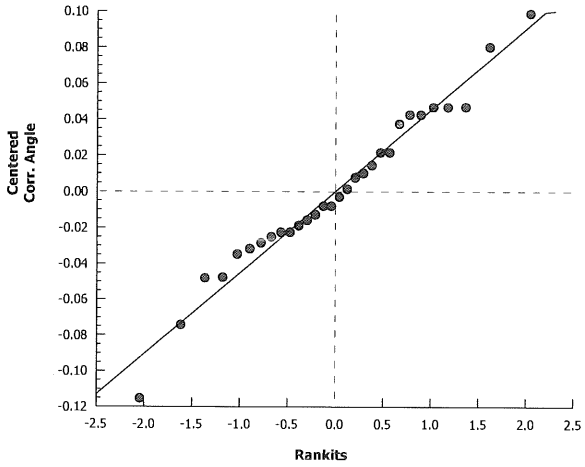
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.145	1.083	1.207	1.159	1.071	1.187	0.02231	4.36%	0.0%
2.5		5	1.155	1.101	1.21	1.133	1.107	1.202	0.01961	3.8%	-0.91%
5		5	1.186	1.094	1.278	1.187	1.071	1.266	0.03323	6.27%	-3.59%
6.06		5	1.167	1.096	1.239	1.159	1.12	1.266	0.02584	4.95%	-1.97%
10		5	1.152	1.121	1.182	1.159	1.12	1.173	0.01085	2.11%	-0.59%
15		5	1.136	1.104	1.167	1.133	1.107	1.173	0.01136	2.24%	0.8%

Echinoid Sperm Cell Fertilization Test 15C — **Nautilus Environmental (CA)**

Analysis ID:	13-2403-4587	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	06 Mar-17 18:51	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

Echinoid Sperm Cell Fertilization Test 15C — **Nautilus Environmental (CA)**

Analysis ID:	07-8967-1481	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	06 Mar-17 18:51	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1693001	1000	Yes	Two-Point Interpolation

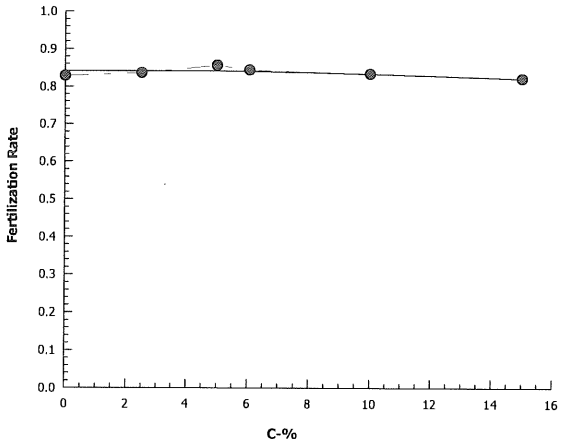
Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC25	>15	N/A	N/A	<6.667	NA	NA
EC50	>15	N/A	N/A	<6.667	NA	NA

Fertilization Rate Summary

			Calculated Variate(A/B)								
C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Lab Control	5	0.828	0.77	0.86	0.01715	0.03834	4.63%	0.0%	414	500
2.5		5	0.836	0.8	0.87	0.01435	0.03209	3.84%	-0.97%	418	500
5		5	0.856	0.77	0.91	0.024	0.05367	6.27%	-3.38%	428	500
6.06		5	0.844	0.81	0.91	0.01749	0.03912	4.63%	-1.93%	422	500
10		5	0.834	0.81	0.85	0.008124	0.01817	2.18%	-0.72%	417	500
15		5	0.822	0.8	0.85	0.008602	0.01924	2.34%	0.72%	411	500

Graphics



CETIS Analytical Report

Echinoid Sperm Cell Fertilization Test 15C — **Nautilus Environmental (CA)**

Analysis ID: 19-8511-9793 **Endpoint:** Fertilization Rate **CETIS Version:** CETISv1.8.7
Analyzed: 06 Mar-17 18:52 **Analysis:** Parametric Bioequivalence-Two Sample **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	TST b	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C*b < T	NA	NA	0.75	3.44%	15	>15	NA	6.667

TST-Welch's t Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		2.5*	11.51	1.895	0.049	7	<0.0001	CDF	Non-Significant Effect
		5*	8.799	2.015	0.075	5	0.0002	CDF	Non-Significant Effect
		6.06*	10.03	1.943	0.06	6	<0.0001	CDF	Non-Significant Effect
		10*	14.69	1.943	0.039	6	<0.0001	CDF	Non-Significant Effect
		15*	13.7	1.895	0.038	7	<0.0001	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.007906956	0.001581391	5	0.6542	0.6612	Non-Significant Effect
Error	0.05801304	0.00241721	24			
Total	0.06592		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	6.506	15.09	0.2600	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9795	0.9031	0.8126	Normal Distribution

Fertilization Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.828	0.7804	0.8756	0.84	0.77	0.86	0.01715	4.63%	0.0%
2.5		5	0.836	0.7962	0.8758	0.82	0.8	0.87	0.01435	3.84%	-0.97%
5		5	0.856	0.7894	0.9226	0.86	0.77	0.91	0.024	6.27%	-3.38%
6.06		5	0.844	0.7954	0.8926	0.84	0.81	0.91	0.01749	4.63%	-1.93%
10		5	0.834	0.8114	0.8566	0.84	0.81	0.85	0.008124	2.18%	-0.72%
15		5	0.822	0.7981	0.8459	0.82	0.8	0.85	0.008602	2.34%	0.72%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.145	1.083	1.207	1.159	1.071	1.187	0.02231	4.36%	0.0%
2.5		5	1.155	1.101	1.21	1.133	1.107	1.202	0.01961	3.8%	-0.91%
5		5	1.186	1.094	1.278	1.187	1.071	1.266	0.03323	6.27%	-3.59%
6.06		5	1.167	1.096	1.239	1.159	1.12	1.266	0.02584	4.95%	-1.97%
10		5	1.152	1.121	1.182	1.159	1.12	1.173	0.01085	2.11%	-0.59%
15		5	1.136	1.104	1.167	1.133	1.107	1.173	0.01136	2.24%	0.8%

CETIS Test Data Worksheet

Report Date: 22 Feb-17 15:34 (p 1 of 1)
Test Code: 1702-5183 11-3910-8922/43E56C3A

Echinoid Sperm Cell Fertilization Test 15C — Nautilus Environmental (CA)

Start Date: 23 Feb-17
End Date: 23 Feb-17
Sample Date: 22 Feb-17

Species: Strongylocentrotus purpuratus
Protocol: EPA/600/R-95/136 (1995)
Material: ~~Seawater~~ Facility Effluent

Sample Code: ~~200C319E~~ 17-0301
Sample Source: IDE Americas, Inc.
Sample Station: Daily M-001

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			51	100	84	2/27/17
			52	100	91	
			53	100	86	
			54	100	82	
			55	100	91	
			56	100	81	
			57	100	87	
			58	100	85	
			59	100	86	
			60	100	77	
			61	100	77	
			62	100	82	
			63	100	85	
			64	100	81	
			65	100	80	
			66	100	82	
			67	100	82	
			68	100	80	
			69	100	85	
			70	100	84	
			71	100	85	
			72	100	81	
			73	100	84	
			74	100	83	
			75	100	86	
			76	100	81	
			77	100	82	
			78	100	84	
			79	100	87	
			80	100	89	

CETIS Test Data Worksheet

Report Date: 22 Feb-17 15:34 (p 1 of 1)
Test Code: 1702-5183 11-3910-8922/43E56C3A

Echinoid Sperm Cell Fertilization Test 15C — **Nautilus Environmental (CA)**

Start Date: 23 Feb-17
End Date: 23 Feb-17
Sample Date: 22 Feb-17
Species: Strongylocentrotus purpuratus
Protocol: EPA/600/R-95/136 (1995)
Material: @ ~~Seawater~~ Facility Effluent
Sample Code: Q18 AC 3/6 ~~200C319E~~ 17-0301
Sample Source: IDE Americas, Inc.
Sample Station: Daily M-001

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	60	100	90	EG 2/23/17
0	LC	2	59			
0	LC	3	51			
0	LC	4	75			
0	LC	5	76			
2.5		1	54			
2.5		2	62			
2.5		3	65	100	92	EG
2.5		4	79			
2.5		5	57			
5		1	55			
5		2	53			
5		3	63	100	91	EG
5		4	61			
5		5	80			
6.06		1	78			
6.06		2	64			
6.06		3	66	100	90	EG
6.06		4	52			
6.06		5	70			
10		1	69			
10		2	73	100	91	EG
10		3	58			
10		4	56			
10		5	67			
15		1	72			
15		2	77	100	89	EG
15		3	68			
15		4	74			
15		5	71			

QC: MM

@ Q18 AC 3/6/17

Marine Chronic Bioassay | **Water Quality Measurements**

Client : IDE | **Test Species:** *S. purpuratus*

Sample ID: Daily M-001 | **Start Date/Time:** 2/23/2017 1442

Sample Log No.: 17- 0301 | **End Date/Time:** 2/23/2017 1522

Dilutions made by: MM | **Test No:** 1702-S183

Analyst: MM

	Initial Readings			
Concentration %	**DO (mg/L)**	**pH (units)**	**Salinity (ppt)**	**Temperature (°C)**
Lab Control	8.4	8.03	33.8	14.7
2.5	8.5	8.04	33.7	14.6
5.0	8.5	8.04	33.7	14.5
6.06	8.5	8.04	Q.S MM 2/23/17 ~~34.8~~ 33.7	14.7
10	8.5	8.03	33.7	14.6
15	8.5	8.03	33.6	14.6

Comments:

QC Check: AC 3/6/17 | **Final Review:** VS 3/7/17

Marine Chronic Bioassay **Echinoderm Sperm-Cell Fertilization Worksheet**

Client: IDE
Sample ID: Daily M-001
Test No.: 1702-5183

Start Date/Time: 2/23/2017 / 1442
End Date/Time: 2/23/2017 / 1522
Species: S. purpuratus
Animal Source: F LOMA
Date Collected: 2/1/17

Tech initials: MM
Injection Time: 1400

Sperm Absorbance at 400 nm: 1.019 (target range of 0.8 - 1.0 for density of $4x10^6$ sperm/ml)

Eggs Counted: 76, 76, 83, 80, 78 Mean: 76.2 X 50 = 3810 eggs/ml

(target counts of 80 eggs per vertical pass on Sedgwick-Rafter slide for a final density of 4000 eggs/ml)

Initial density: 3810 eggs/ml = (A) dilution factor
Final density: 4000 eggs/ml - 1.0 part egg stock
[ ] parts seawater

egg stock (A) ml
seawater ml

Prepare the embryo stock according to the calculated dilution factor. For example, if the dilution factor is 2.25, use 100 ml of existing stock (1 part) and 125 ml of dilution water (1.25 parts).

	Sperm:Egg Ratio							
Rangefinder Test:	**2000:1**	**1600:1**	**1200:1**	**800:1**	**400:1**	**200:1**	**100:1**	**50:1**
ml Sperm Stock	50	40	30	20	10	5.0	2.5	1.25
ml Seawater	0.0	10	20	30	40	45	47.5	48.75

	Time	Rangefinder Ratio:	Fert.	Unfert.
Sperm Added (100 µl):	1413	50	81/71	19/29
Eggs Added (0.5 ml):	1423	100	98/98	2/2
Test Ended:	1433	200	100	0
		—	—	—

NOTE: Choose a sperm-to-egg ratio that results in fertilization between 80 and 90 percent. If more than one concentration is within this range, choose the ratio closest to 90 percent unless professional judgment dictates consideration of other factors (e.g., organism health, stage of reproductive season, site conditions).

Definitive Test Sperm:Egg Ratio Used: 75:1

	Time		Fert.	Unfert.
Sperm Added (100 µl):	1442	QC1	90	10
Eggs Added (0.5 ml):	1502	QC2	89	11
Test Ended:	1522	Egg Control 1	0	100
		Egg Control 2	0	100

Comments: (A) No dilution needed

QC Check: AC 3/6/17 Final Review: 3/7/17

Appendix B

Sample Receipt Information

Nautilus Environmental
4340 Vandever Avenue
San Diego, CA 92120

Client: IDE
Sample ID: Daily M-601
Test ID No(s).: 1702-S183 + S184

Sample (A, B, C):	A			
Log-in No. (17-xxxx):	0301			
Sample Collection Date & Time:	2/22/17 1200			
Sample Receipt Date & Time:	2/22/17 1325			
Number of Containers & Container Type:	2.4L cubes			
Approx. Total Volume Received (L):	8L			
Check-in Temperature (°C)	3.2			
Temperature OK? [1]	(Y) N	Y N	Y N	Y N
DO (mg/L)	8.0			
pH (units)	7.80			
Conductivity (µS/cm)	—			
Salinity (ppt)	31.5			
Alkalinity (mg/L) [2]	111			
Hardness (mg/L) [2,3]	—			
Total Chlorine (mg/L)	<0.02			
Technician Initials	AP			

Test Performed: Urchin Fert. **Control/Dilution Water:** 8:2 / (Lab SW) / Lab ART Other: _____
Alkalinity: 118 Hardness or Salinity: 34 ppt
Additional Control? Y (N) = _____ Alkalinity: _____ Hardness or Salinity: _____

Test Performed: _____ **Control/Dilution Water:** 8:2 / Lab SW / Lab ART Other: _____
Alkalinity: _____ Hardness or Salinity: _____
Additional Control? Y N = _____ Alkalinity: _____ Hardness or Salinity: _____

Test Performed: _____ **Control/Dilution Water:** 8:2 / Lab SW / Lab ART Other: _____
Alkalinity: _____ Hardness or Salinity: _____
Additional Control? Y N = _____ Alkalinity: _____ Hardness or Salinity: _____

Notes: [1] Temperature of sample should be 0-6°C, if received more than 24 hours past collection time.
[2] mg/L as CaCO3, [3] Measured for freshwater samples only, NA = Not Applicable

Additional Comments: _____

Sample Check-In Information

Sample Description: colorless, clear, odorless, no debris

COC Complete (Y/N)?
A ✓ B____ C____

Filtration? Y (N)
Pore Size: _____
Organisms or Debris

Salinity Adjustment? Y (N)
Test: Source: Target ppt:
Test: Source: Target ppt:
Test: Source: Target ppt:

pH Adjustment? Y (N)

	A	B	C
Initial pH:			
Amount of HCl added:			
Final pH:			

Cl_2 Adjustment? Y (N)

	A	B	C
Initial Free Cl_2:			
STS added:			
Final Free Cl_2:			

Sample Aeration? Y (N)

	A	B	C
Initial D.O.			
Duration & Rate			
Final D.O.			

Subsamples for Additional Chemistry Required? Y (N)
NH3 Other _____
Tech Initials A____ B____ C____

QC Check: AC 3/6/17
Final Review: [illegible] 3/7/17

Appendix C

Chain-of-Custody Form

IDE Technologies

CDP laoratory:_____
Entahlpy Laboratory:____
WECK Laboratory:_____
Nautilus:____X_____
AIM:_____
Other:_____

Turn Around Time
Normal:____X_____
RUSH (24 hr):_____
3 Days:_____
5 Days:_____
??? Days_____

Project Name: Daily NPDES Monitoring Project Manager: Peter Shen Contact Information: (760) 201-7777

Comments:

14 hour composite sampled via autosampler by a series of consecutive grabs at one hour intervals. Sample collected during plant off-spec (pretreatment and RO in operation) to fulfill daily NPDES requirement. Sample is to be run undiluted. Conductivity: 48.55, TDS: 31.09. Start: 2200, End 1100. VH 2/22/2017

Glass=G Plastic=P

Yes=Y No=N Acid=A Base=B

Drinking Water=DW Seawater=SW Soil=S

ANALYSES

Sample ID	Date	Time	Sample Type	Preservative ?	Container Type	Purple Urchin Chronic Fertilization								NOTES:
Daily M-001 (17-1133)	2/21-22/2017	22:00-12:00	14 hr Composite	N	2L cubi	X								KE/VH

Relinquished By:	Date:	Time:		Received By:	Time:	Sample Condition Upon Receipt:
Kevin Cheung	2/22/17	1230		2/22/17	1930	☒ Iced ☐ Ambient or 3.2 °C
	2/22/17	1:25PM		Adrienne Cabe 2/22/17	1325	☐ Iced ☐ Ambient or _____ °C

Nautilus Log. in #: 17-0301

Appendix D

Reference Toxicant Test Data and Statistical Analyses

CETIS Summary Report

Echinoid Sperm Cell Fertilization Test 15C — **Nautilus Environmental (CA)**

Batch ID:	08-4171-0053	**Test Type:**	Fertilization	**Analyst:**	
Start Date:	23 Feb-17 14:42	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural Seawater
Ending Date:	23 Feb-17 15:22	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	40m	**Source:**	Pt. Loma	**Age:**	

Sample ID:	16-9625-0773	**Code:**	651ABB95	**Client:**	Internal
Sample Date:	23 Feb-17	**Material:**	Copper chloride	**Project:**	
Receive Date:	23 Feb-17	**Source:**	Reference Toxicant		
Sample Age:	15h	**Station:**	Copper Chloride		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
15-2282-2936	Fertilization Rate	<10	10	NA	8.43%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
20-4334-6940	Fertilization Rate	EC50	23.77	21	26.92		Trimmed Spearman-Kärber

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
15-2282-2936	Fertilization Rate	Control Resp	0.842	0.7 - NL	Yes	Passes Acceptability Criteria
20-4334-6940	Fertilization Rate	Control Resp	0.842	0.7 - NL	Yes	Passes Acceptability Criteria
15-2282-2936	Fertilization Rate	PMSD	0.08428	NL - 0.25	No	Passes Acceptability Criteria

Fertilization Rate Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	5	0.842	0.8137	0.8703	0.82	0.88	0.0102	0.0228	2.71%	0.0%
10		5	0.542	0.4618	0.6222	0.49	0.65	0.02888	0.06458	11.91%	35.63%
20		5	0.492	0.3741	0.6099	0.39	0.61	0.04247	0.09497	19.3%	41.57%
40		5	0.276	0.1915	0.3605	0.16	0.33	0.03043	0.06804	24.65%	67.22%
80		5	0.004	0	0.0108	0	0.01	0.002449	0.005477	136.9%	99.52%
160		5	0.002	0	0.007553	0	0.01	0.002	0.004472	223.6%	99.76%

Fertilization Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.88	0.84	0.83	0.82	0.84
10		0.49	0.65	0.55	0.5	0.52
20		0.42	0.57	0.61	0.47	0.39
40		0.32	0.33	0.16	0.28	0.29
80		0	0.01	0	0.01	0
160		0	0	0	0	0.01

Echinoid Sperm Cell Fertilization Test 15C — **Nautilus Environmental (CA)**

Analysis ID: 15-2282-2936 **Endpoint:** Fertilization Rate **CETIS Version:** CETISv1.8.7
Analyzed: 02 Mar-17 12:00 **Analysis:** Parametric-Control vs Treatments **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	8.43%	<10	10	NA	

Dunnett Multiple Comparison Test

Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		10*	8.696	2.362	0.091	8	<0.0001	CDF	Significant Effect
		20*	10.01	2.362	0.091	8	<0.0001	CDF	Significant Effect
		40*	15.91	2.362	0.091	8	<0.0001	CDF	Significant Effect
		80*	28.37	2.362	0.091	8	<0.0001	CDF	Significant Effect
		160*	28.63	2.362	0.091	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	4.855814	0.9711628	5	261.9	<0.0001	Significant Effect
Error	0.08900392	0.003708497	24			
Total	4.944818		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	11.84	15.09	0.0371	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9638	0.9031	0.3860	Normal Distribution

Fertilization Rate Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.842	0.8137	0.8703	0.84	0.82	0.88	0.0102	2.71%	0.0%
10		5	0.542	0.4618	0.6222	0.52	0.49	0.65	0.02888	11.91%	35.63%
20		5	0.492	0.3741	0.6099	0.47	0.39	0.61	0.04247	19.3%	41.57%
40		5	0.276	0.1915	0.3605	0.29	0.16	0.33	0.03043	24.65%	67.22%
80		5	0.004	0	0.0108	0	0	0.01	0.002449	136.9%	99.52%
160		5	0.002	0	0.007553	0	0	0.01	0.002	223.6%	99.76%

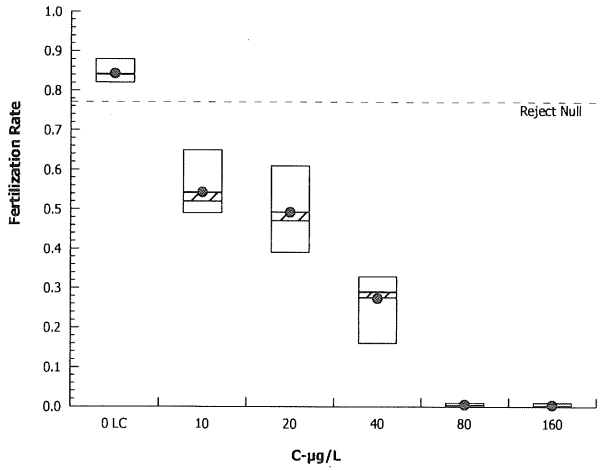
Angular (Corrected) Transformed Summary

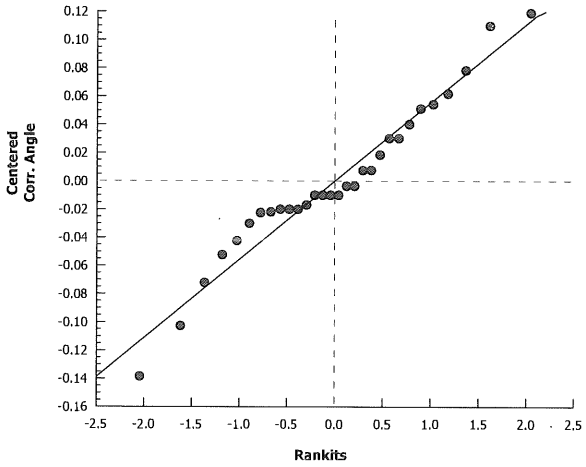
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.163	1.123	1.203	1.159	1.133	1.217	0.01443	2.78%	0.0%
10		5	0.8279	0.7465	0.9093	0.8054	0.7754	0.9377	0.02932	7.92%	28.8%
20		5	0.7774	0.6587	0.8961	0.7554	0.6745	0.8963	0.04276	12.3%	33.15%
40		5	0.5502	0.45	0.6504	0.5687	0.4115	0.6119	0.03609	14.67%	52.68%
80		5	0.07008	0.03598	0.1042	0.05002	0.05002	0.1002	0.01228	39.19%	93.97%
160		5	0.06005	0.0322	0.0879	0.05002	0.05002	0.1002	0.01003	37.35%	94.84%

Echinoid Sperm Cell Fertilization Test 15C — **Nautilus Environmental (CA)**

Analysis ID:	15-2282-2936	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	02 Mar-17 12:00	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

Echinoid Sperm Cell Fertilization Test 15C — **Nautilus Environmental (CA)**

Analysis ID:	20-4334-6940	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	02 Mar-17 12:01	**Analysis:**	Trimmed Spearman-Kärber	**Official Results:**	Yes

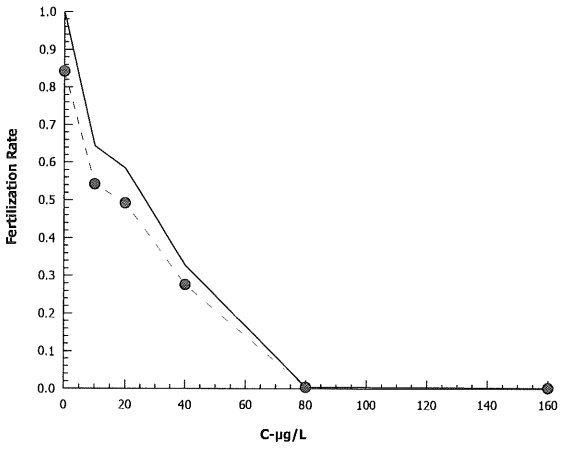
Trimmed Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.158	35.63%	1.376	0.02697	23.77	21	26.92

Fertilization Rate Summary

			Calculated Variate(A/B)								
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Lab Control	5	0.842	0.82	0.88	0.0102	0.0228	2.71%	0.0%	421	500
10		5	0.542	0.49	0.65	0.02888	0.06458	11.91%	35.63%	271	500
20		5	0.492	0.39	0.61	0.04247	0.09497	19.3%	41.57%	246	500
40		5	0.276	0.16	0.33	0.03043	0.06804	24.65%	67.22%	138	500
80		5	0.004	0	0.01	0.002449	0.005477	136.9%	99.52%	2	500
160		5	0.002	0	0.01	0.002	0.004472	223.6%	99.76%	1	500

Graphics

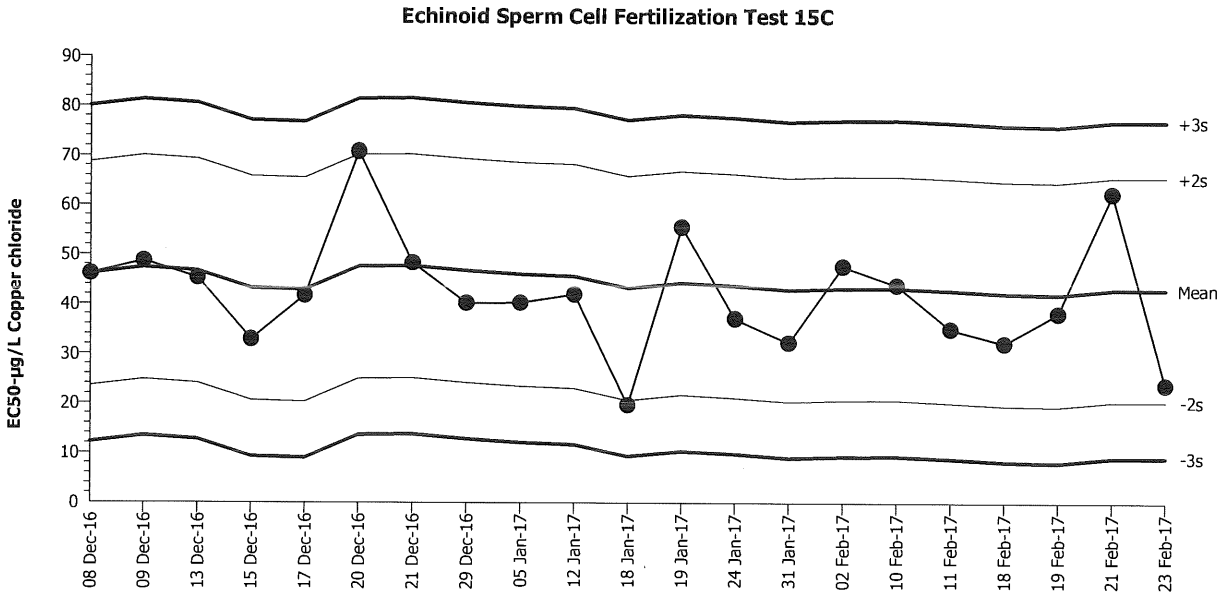


CETIS QC Plot

Report Date: 02 Mar-17 12:03 (1 of 1)

Echinoid Sperm Cell Fertilization Test 15C — **Nautilus Environmental (CA)**

Test Type:	Fertilization	**Organism:**	Strongylocentrotus purpuratus (Purpl	**Material:**	Copper chloride
Protocol:	EPA/600/R-95/136 (1995)	**Endpoint:**	Fertilization Rate	**Source:**	Reference Toxicant-REF



Mean:	43	**Count:**	20	**-2s Warning Limit:**	20.38	**-3s Action Limit:**	9.07
Sigma:	11.31	**CV:**	26.30%	**+2s Warning Limit:**	65.62	**+3s Action Limit:**	76.93

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2016	Dec	8	15:04	46.14	3.141	0.2777			11-3755-6520	02-1834-2654
2			9	16:26	48.68	5.678	0.502			11-3406-8076	17-4460-9811
3			13	15:11	45.26	2.259	0.1998			10-6683-7365	04-6270-7422
4			15	17:33	32.87	-10.13	-0.8955			01-7454-5472	05-8893-7899
5			17	15:22	41.72	-1.282	-0.1134			08-9842-8510	01-1488-1013
6			20	15:17	70.85	27.85	2.462	(+)		16-6092-1425	02-2928-0983
7			21	12:12	48.26	5.264	0.4654			14-5051-2365	16-1479-8388
8			29	16:22	40.16	-2.837	-0.2509			17-0784-9661	08-0208-3856
9	2017	Jan	5	14:34	40.21	-2.788	-0.2465			04-1406-8806	15-3393-3643
10			12	17:54	41.95	-1.051	-0.09297			14-8351-4083	12-3796-8723
11			18	15:19	19.65	-23.35	-2.064	(-)		08-8914-3626	00-6318-6085
12			19	18:19	55.59	12.59	1.113			09-5789-7052	01-7604-4546
13			24	15:37	37.1	-5.904	-0.522			18-0430-6783	19-8873-5804
14			31	15:00	32.26	-10.74	-0.9497			14-3391-7268	12-6240-4784
15		Feb	2	16:50	47.66	4.662	0.4122			10-8641-2413	14-8698-1832
16			10	15:05	43.85	0.8473	0.07492			18-1100-4857	17-9587-0468
17			11	13:32	35.02	-7.975	-0.7051			21-3415-8415	20-8117-2853
18			18	14:43	32.15	-10.85	-0.9591			15-8602-9109	03-0004-4079
19			19	16:00	38.18	-4.824	-0.4265			04-9561-8356	16-1145-1366
20			21	11:42	62.44	19.44	1.719			15-6576-1294	19-2980-3814
21			23	14:42	23.77	-19.23	-1.7			07-0628-7264	20-4334-6940

CETIS Test Data Worksheet

Report Date: 21 Feb-17 12:46 (p 1 of 1)
Test Code: 07-0628-7264/170223sprt

Echinoid Sperm Cell Fertilization Test 15C — **Nautilus Environmental (CA)**

Start Date: 23 Feb-17
End Date: 23 Feb-17
Sample Date: 23 Feb-17
Species: Strongylocentrotus purpuratus
Protocol: EPA/600/R-95/136 (1995)
Material: Copper chloride
Sample Code: (A) ~~851ABB95~~ 170223sprt
Sample Source: Reference Toxicant
Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
			1	100	65	2/28/17
			2	100	55	
			3	100	28	
			4	100	42	
			5	100	1	
			6	100	0	
			7	100	0	
			8	100	0	
			9	100	61	
			10	100	0	
			11	100	57	
			12	100	39	
			13	100	49	
			14	100	83	
			15	100	0	
			16	100	50	
			17	100	47	
			18	100	84	
			19	100	84	
			20	100	33	
			21	100	32	
			22	100	29	
			23	100	82	
			24	100	16	
			25	100	52	
			26	100	88	
			27	100	1	
			28	100	1	
			29	100	0	
			30	100	0	

(A) CHQ18 2/24/17

SG

Analyst: ~~CH~~ CHQ18 2/24/17 QA: KFP 3/8/17

CETIS Test Data Worksheet

Report Date: 21 Feb-17 12:44 (p 1 of 1)
Test Code: 07-0628-7264/170223sprt

Echinoid Sperm Cell Fertilization Test 15C — **Nautilus Environmental (CA)**

Start Date: 23 Feb-17
End Date: 23 Feb-17
Sample Date: 23 Feb-17
Species: Strongylocentrotus purpuratus
Protocol: EPA/600/R-95/136 (1995)
Material: Copper chloride
Sample Code: ~~651ABB95~~ 170223sprt
Sample Source: Reference Toxicant
Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	26			
0	LC	2	18			
0	LC	3	14	100	94	MM 2/23/17
0	LC	4	23			
0	LC	5	19			
10		1	13			
10		2	1			
10		3	2	100	74	MM 2/23/17
10		4	16			
10		5	25			
20		1	4			
20		2	11			
20		3	9	100	63	MM 2/23/17
20		4	17			
20		5	12			
40		1	21			
40		2	20			
40		3	24	100	36	MM 2/23/17
40		4	3			
40		5	22			
80		1	8			
80		2	28			
80		3	6	100	0	MM 2/23/17
80		4	5			
80		5	15			
160		1	29			
160		2	7			
160		3	30			
160		4	10	100	0	MM 2/23/17
160		5	27			

QC MM

ABAO QA 2/22/17

Marine Chronic Bioassay | **Water Quality Measurements**

Client : Internal | **Test Species:** *S. purpuratus*

Sample ID: $CuCl_2$ | **Start Date/Time:** 2/23/2017 1442

Test No: 170223sprt | **End Date/Time:** 2/23/2017 1522

Dilutions made by: MM

High conc. made (µg/L):	160
Vol. Cu stock added (mL):	8.2
Final Volume (mL):	500
Cu stock concentration (µg/L):	9800

Analyst: MM

	Initial Readings			
Concentration (µg/L)	**DO (mg/L)**	**pH (units)**	**Salinity (ppt)**	**Temperature (°C)**
Lab Control	8.5	8.04	33.8	14.8
10	8.4	8.05	33.8	14.8
20	8.4	8.06	33.8	14.8
40	8.4	8.06	33.7	14.9
80	8.2	8.07	33.5	14.9
160	8.4	8.08	33.5	14.9

Comments:

QC Check: KB 3/2/17 | **Final Review:** KTP 3/8/17

Nautilus Environmental. 4340 Vandever Avenue. San Diego, CA 92120.

Marine Chronic Bioassay **Echinoderm Sperm-Cell Fertilization Worksheet**

Client: Internal
Sample ID: CuCl2
Test No.: 170223 spf

Start Date/Time: 2/23/2017 / 1442
End Date/Time: 2/23/2017 / 1522
Species: S. purpuratus
Animal Source: PT LOMA
Date Collected: 2/1/17

Tech initials: MM
Injection Time: 1400

Sperm Absorbance at 400 nm: 1.019 (target range of 0.8 - 1.0 for density of $4x10^6$ sperm/ml)

Eggs Counted: 70, 70, 83, 80, 78 Mean: 76.2 X 50 = 3810 eggs/ml

(target counts of 80 eggs per vertical pass on Sedgwick-Rafter slide for a final density of 4000 eggs/ml)

Initial density: 3810 eggs/ml = (A) dilution factor
Final density: 4000 eggs/ml - 1.0 part egg stock
[ ] parts seawater

egg stock (A) ml
seawater ml

Prepare the embryo stock according to the calculated dilution factor. For example, if the dilution factor is 2.25, use 100 ml of existing stock (1 part) and 125 ml of dilution water (1.25 parts).

	Sperm:Egg Ratio							
Rangefinder Test:	**2000:1**	**1600:1**	**1200:1**	**800:1**	**400:1**	**200:1**	**100:1**	**50:1**
ml Sperm Stock	50	40	30	20	10	5.0	2.5	1.25
ml Seawater	0.0	10	20	30	40	45	47.5	48.75

	Time	Rangefinder Ratio:	Fert.	Unfert.
Sperm Added (100 µl):	1413	50	81/71	19/29
Eggs Added (0.5 ml):	1423	100	98/98	2/2
Test Ended:	1433	200	100	0

NOTE: Choose a sperm-to-egg ratio that results in fertilization between 80 and 90 percent. If more than one concentration is within this range, choose the ratio closest to 90 percent unless professional judgment dictates consideration of other factors (e.g., organism health, stage of reproductive season, site conditions).

Definitive Test Sperm:Egg Ratio Used: 75:1

	Time		Fert.	Unfert.
Sperm Added (100 µl):	1442	QC1	90	10
Eggs Added (0.5 ml):	1502	QC2	89	11
Test Ended:	1522	Egg Control 1	0	100
		Egg Control 2	0	100

Comments: (A) No dilution needed

QC Check: VB 3/2/17 Final Review: KFP 3/8/17

Appendix E

Qualifier Codes

Nautilus Environmental

Glossary of Qualifier Codes:

Q1 - Temperatures out of recommended range; corrective action taken and recorded in Test Temperature Correction Log

Q2 - Temperatures out of recommended range; no action taken, test terminated same day

Q3 - Sample aerated prior to initiation or renewal due to dissolved oxygen (D.O.) levels below 6.0 mg/L

Q4 - Test aerated; D.O. levels dropped below 4.0 mg/L

Q5 - Test initiated with aeration due to an anticipated drop in D.O.

Q6 - Airline obstructed or fell out of replicate and replaced; drop in D.O. occurred

Q7 - Salinity out of recommended range

Q8 - Spilled test chamber/ Unable to recover test organism(s)

Q9 - Inadequate sample volume remaining, 50% renewal performed

Q10 - Inadequate sample volume remaining, no renewal performed

Q11 - Sample out of holding time; refer to QA section of report

Q12 - Replicate(s) not initiated; excluded from data analysis

Q13 - Survival counts not recorded due to poor visibility or heavy debris

Q14 - D.O. percent saturation was checked and was ≤ 110%

Q15 - Did not meet minimum test acceptability criteria. Refer to QA section of report.

Q16 - Percent minimum significant difference (PMSD) was below the lower bound limit for acceptability. This indicates that statistics may be over-sensitive in detecting a difference from the control due to low variability in the data set.

Q17 - Percent minimum significant difference (PMSD) was above the upper bound limit for acceptability. This indicates that statistics may be under-sensitive in detecting a difference from the control due to high variability in the data set.

Q18 - Incorrect Entry

Q19 - Illegible Entry

Q20 - Miscalculation

Q21 - Other (provide reason in comments section)

Q22 - Greater than 10% mortality observed upon receipt and/or in holding prior to test initiation. Organisms acclimated to test conditions at Nautilus and ultimately deemed fit to use for testing.

Q23 - Test organisms received at a temperature greater than 3°C outside the recommended test temperature range. However, due to age-specific protocol requirements and/or sample holding time constraints, the organisms were used to initiate tests upon the day of arrival. Organisms were acclimated to the appropriate test conditions upon receipt and prior to test initiation.

Q24 - Test organisms received at salinity greater than 3 ppt outside of the recommended test salinity range. However, due to age-specific protocol requirements and/or sample holding time constraints, the organisms were used to initiate tests upon the day of arrival. Organisms were acclimated to the appropriate test conditions upon receipt and prior to test initiation.